Study of overlapping of reagent zones in LOV format and application to enzymatic assays

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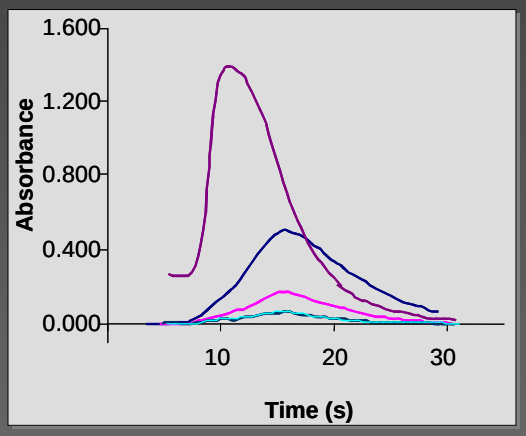
In sequential injection systems, mixing is based on the degree of overlapping between adjacent zones. Therefore, efficient mixing might be difficult to achieve when various solutions are concerned. That can be the case of enzymatic assays involving sample, enzyme, buffer and frequently cofactor solutions.

One way to overcome this problem is the use of reduced volumes of solutions. In this work the lab-on-valve LOV-FIAlab-3500 system was used.

STUDY OF OVERLAPPING OF REAGENT ZONES IN LOV



Recorded peak profiles obtained by the injection of bromothimol blue solution (24 mg L^{-1}) and corresponding aspiration sequence.

REPEATABILITY

Reagent zones	50 µL buffer	15 µL sample	5 µL ADH	5 µL NAD^+	100 µL buffer
RSD (%) (n=10)	< 0.7	< 1.0	< 2.9	< 3.0	< 0.4

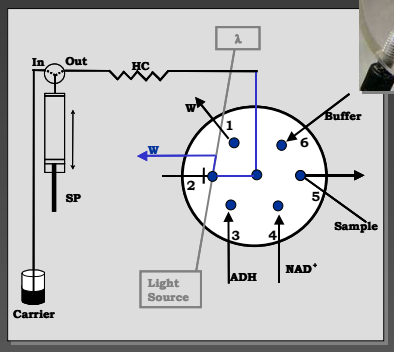
FIGURES OF MERIT OF THE DEVELOPED SYSTEM

Parameter	Value
Reagent consumption	
ADH	0.28 U/assay
NAD +	0.066 mg/assay
Sample	71 µL/determination
Waste production	1.2 mL/assay
Determination rate	28 h^{-1}
Application zone	0 to 0.8% (v/v)
RSD% (n=5)	1.0% (9.1% v/v)
	6.3% (12.2% v/v)
	0.7% (11.1% v/v)
	1.0% (10.4% v/v)

APPLICATION TO ENZYMATIC ASSAYS

$$\text{Ethanol} + NAD^+ \xrightarrow{ADH} \text{Acetaldehyde} + NADH + H^+$$

FLOW MANIFOLD



LOV-SIA manifold for the determination of ethanol; ADH, Alcohol dehydrogenase; NAD^+, cofactor 20 mM; Buffer, phosphate buffer pH 9.5; W, waste; Carrier, water; SP, syringe pump (2.5 mL); HC, holding coil; λ, Diode-array spectrophotometer.

FLOW PROTOCOL SEQUENCE

Description	Volume (µL)	Flow rate (µL/s)	Selection Valve position	Syringe Pump
Aspirate carrier	1000	100	-	In
Aspirate buffer	50	80	6	Out
Aspirate sample	15	25	5	Out
Aspirate ADH	5	25	3	Out
Aspirate NAD^+	5	25	4	Out
Aspirate buffer	100	25	6	Out
Send to flow cell	Syringe pump empty	15	2	Out

RESULTS OBTAINED FOR THE ANALYSIS OF DIFFERENT WINE TYPES

Wine sample	Ref. Met. [a] [b] % ethanol (v/v)	LOV [c] % ethanol (v/v)	R.D.,% [d]
Red table wine	9.3 ± 0.1	9.1 ± 0.1	-2
Red table wine	12.4 ± 0.1	12.2 ± 0.8	-1.7
Red table wine	11.1 ± 0.1	11.1 ± 0.1	0.1
White table wine	10.2 ± 0.1	10.4 ± 0.1	1.6

[a] *Office International de la Vigne et du Vin (OIV) (1978) Recueil des Méthodes Internationales d'Analyse des Vins et des Moûts, OIV, Paris pp. 61-62*
[b] *mean and accepted precision for n=3*
[c] mean and standard deviation for (n=4)
[d] R.D. – Relative deviation

S. Vidigal and I.Tóth thank Fundação para a Ciência e Tecnologia (FCT) and FSE (III Quadro Comunitário) for the grants SFRH/BD/23040/2005 and SFRH/BPD/5631/2001, respectively.